SPATIAL LIGHT MODULATOR APPARATUS AND METHOD

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001]   This application claims the benefit of Provisional Application No.

60/514,888 filed in the U.S.P.T.O. on October 28, 2003. The present application

incorporates the disclosure of this provisional application by reference.

FIELD OF THE INVENTION

[0002]   This invention relates to microscope systems that utilize spatial light

modulators for illuminating a specimen, for example, fluorescent microscopes.

BACKGROUND OF THE INVENTION

[0003]   Microscopes are well-established instruments for observing minute

biological structures and processes. This field of art includes fluorescent

microscopy, which allows a user to achieve a high degree of target specificity and

image contrast for biological specimens. Conventional fluorescent microscopes

employ the basic illumination scheme shown in Figure 1. In Figure 1, a viewing

device 10 (such as a Charged Coupled Device (CCD) or camera or eye) is used to

observe a biological sample 22 which rests upon stage 20. The viewing device 10

includes a viewing path through an image forming tube lens 26, an emission filter

34, a dichroic mirror 30, and an objective lens 24.

[0004]   A lamp 40 emits an excitation light 50 that has passed through the

collector 32 and which then passes through the conjugate image plane located at

the field diaphragm 42. The excitation light 50 continues through the projection

tube lens 28 and an excitation filter 36. The excitation light 50 is of a wavelength

that is reflected downward by the dichroic mirror 30, after which it passes through

objective 24 to thereby reach sample 22 where the excitation light 50 stimulates a

fluorescent marker in the sample 22. The sample 22, thus excited, emits a

fluorescent emission which is of a wavelength permitted to pass through the

dichroic mirror 30, and which is viewed by the viewing device 10.

[0005]   Known methods for illuminating a biological specimen in fluorescent

microscopy utilize an even excitation illumination across the entire field of view.

For instance, Kohler illumination applies an even excitation light across an entire

sample. Problems arise from these known systems of illumination, however, as

there are situations where it may be better to vary the intensity of illumination

over different regions of the specimen.

[0006]   For instance, during live cell imaging only a portion of the field of view is

of interest and unnecessary exposure of light to living tissue in the remainder of

the field can result in photo-toxicity and/or photo-bleaching. An additional

problem occurs when a bright object is located in front or in back of a structure of

interest, making it impossible to clearly view the object of interest.

[0007]   Photo-toxicity is a particularly troubling problem in live cell fluorescence

microscopy. First, fluorescence microscopy requires very intense excitation

illumination that is typically many orders of magnitude brighter than that used for

conventional brightfield microscopy. Photo-toxicity due to an intense excitation

illumination light is often the ultimate limitation in live cell fluorescence imaging. Secondly, all fluorescent dyes produce toxic free radicals upon illumination with excitation light.

[0008]   The current invention minimizes photo-toxicity and photo-bleaching through control of the excitation illumination, for instance, by modulating the intensity of the excitation light through at least one of spatial modulation and temporal modulation. In spatial modulation, different regions of the specimen are simultaneously illuminated with different intensities of excitation light based on the needs and interests of the observer. In temporal modulation, different regions of the specimen are scanned by an excitation light of varying intensity over a time period.

[0009]   Electronic image sensors and detectors have replaced photographic cameras as the primary means to record images in wide-field fluorescence microscopy. An inherent weakness of current digital detectors is a limited ability to record images with high variations in brightness. This becomes a serious problem when sub-regions of a sample are much brighter than the regions of interest. The bright regions saturate the detector while the regions of interest are inadequately illuminated. These problems are discussed below in relation to Figs. 2A – 2C.

[0010]   Image quality depends upon the proper biasing of illumination brightness so as to stay within the optimal detection range of the CCD detector. Under-excitation results in a poor signal-to-noise ratio. An example of under-excitation

is shown in Fig. 2A, where the image of the sample was acquired under insufficient illumination. Other problems result from over-excitation. As shown in Fig. 2C, the sample being viewed was over-excited, resulting in pixel saturation at the CCD. Note that the image is distorted, leaving no plausible manner in which to perform quantitative analysis.

[0011]   As shown in Fig. 2B and so long as the sample being viewed has relatively low variations in brightness between various portions of the sample, it should be possible to find a proper level of excitation illumination that can produce a qualitatively pleasing and quantitatively accurate image. However, with current microscopes, and when a sample's emitted brightness possesses moderate-to-high variability, it has been heretofore impossible to select a single proper level of excitation illumination (*i.e.*, exposure duration) that will produce optimized imaging for each aspect of a sample being viewed.

SUMMARY OF THE INVENTION

[0012]   The present invention provides an apparatus and method for minimizing photo-toxicity during live cell imaging. Secondly, the present invention enables fluorescent samples with widely varying brightness to be optimally recorded with electronic detectors by spatially modulating the intensity of illumination. The degree of modulation at each spatial location is recorded and can be subsequently used to reconstruct a quantitatively accurate image that is not distorted by the limitations of the electronic detector. Thirdly, the present invention further relates

to a confocal mask using spatial light modulation and including computer

readable means to implement the above. Details of the present invention are

further described in the Detailed Description of Embodiments section, below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013]   The invention, both as to its organization and manner of operation, may be

further understood by reference to the drawings that include Figures 1 – 18C,

taken in connection with the following detailed description of exemplary

embodiments.

[0014]   Fig. 1. is an illustration of a prior art excitation light illumination system

capable of being used with a fluorescent microscope;

[0015]   Fig. 2A is an image of a dendrite of a live green fluorescent protein (GFP)

labeled neuron that has not received sufficient excitation illumination;

[0016]   Fig. 2B is the same dendrite of Fig. 2A, but which has received sufficient

excitation illumination;

[0017]   Fig. 2C is the same dendrite of Figs. 2A and 2B, but taken with excessive

excitation illumination;

[0018]   Fig. 3 is an embodiment of the present invention including a spatial light

modulator enabling discriminate excitation illumination modulation on a region-

by-region basis of the sample under observation;

[0019]   Fig. 4A is an image taken of two spheres of differing brightness, wherein

the first, dimmer sphere cannot be clearly detected above background noise while

the brighter, second sphere has reached saturation;

[0020]   Fig. 4B is an image taken of the same two spheres of Fig. 4A, except

illumination has been increased until the dim sphere can be clearly observed,

resulting in the bright sphere surpassed detector saturation;

[0021]   Fig. 4C is an image of the same two spheres using the discriminate

stimulating light modulation of the present invention, thereby allowing both

spheres to be clearly detected and amenable for quantitatively analysis;

[0022]   Fig. 4D is a graphical representation of the luminescence produced by the

two spheres in Fig. 4A;

[0023]   Fig. 4E is a graphical representation of the luminescence produced by the

two spheres in Fig. 4B;

[0024]   Fig. 4F is a graphical representation of the luminescence produced by the

two spheres in Fig. 4C;

[0025]   Fig. 5A is an illustration of the present invention including an LCD

mounted on a slider and connected to an LCD drive controller by a ribbon cable;

[0026]   Fig. 5B is an illustration of the present invention including the slider of

Fig. 5A mounted in the field diaphragm slot of the epifluorescence unit of a

Nikon microscope;

[0027]   Fig. 6 is an image of components of an embodiment of the present

invention including a liquid crystal display and a digital micro-mirror device;

[0028]   Fig. 7 illustrates an schematic of the invention including mechanical and electrical components separate from a microscope;

[0029]   Fig. 8 illustrates an embodiment of the invention including the sub-components of an LCD projector/controller;

[0030]   Fig. 9A illustrates an image projected by the LCD of the present invention including a calibration technique where two points are projected by the LCD projection array;

[0031]   Fig. 9B illustrates a co-registration of the two points shown in Fig. 9A with a CCD collection array;

[0032]   Figs. 10A-D illustrate examples of constructed field iris masks according to an embodiment of the invention;

[0033]   Fig. 11A illustrates a linear graph of intensity of detected luminance at an image detector as compared to the luminance intensity of a sample being viewed without any type of masking;

[0034]   Fig. 11B illustrates a graph of intensity of detected luminance at an image detector as compared to the luminance intensity of a sample being viewed using a logarithmic density transfer function as an automated mask;

[0035]   Fig. 11C illustrates a graph of intensity of detected luminance at an image detector as compared to the luminance transmittance of a sample being viewed using a logarithmic density transfer function as an automated mask;

[0036]   Fig. 12A illustrates a conventional method of viewing a sample with more than one plane of focus, causing an out-of-focus image;

[0037]   Fig. 12B illustrates an embodiment of the invention with the ability to

mask an out-of-focus structure without obscuring the in-focus structure using

confocal masking;

[0038]   Fig. 13A illustrates an embodiment of the invention including a reference

image of a set of white bars against a black background that is useful for

calibrating contrast, resolution and aberration;

[0039]   Fig. 13B illustrates an embodiment of the invention where one white bar

from Fig. 13A has been scanned to thereby allow a measure of absolute intensity,

resolution and aberration (as detected by each CCD pixel);

[0040]   Fig. 14A illustrates a top view of an embodiment of the invention

including a sliding fork that is capable of moving an LCD to thereby adjust the

focus of a projected image;

[0041]   Fig. 14B illustrates a side view of the sliding fork shown in Fig. 14A,

wherein the sliding fork allows a projected image to be adjusted for focus;

[0042]   Fig. 15 illustrates an embodiment of the invention including the capability

to change the focal length of the illumination tube lens thereby allowing changes

in the relative magnification of the pattern displayed by the spatial light modulator

and that detected by the image sensor;

[0043]   Fig. 16 illustrates an embodiment of the invention including the use of a

Digital Micro-Mirror Device (DMD);

[0044]   Fig. 17A is an image taken using a conventional homogenous epi-

illumination system of a live neuron expressing green fluorescent protein (GFP);

[0045]   Fig. 17B is an image of the same neuron shown in Fig. 17A, but taken

using the excitation light modulation apparatus of the present invention, thereby

allowing for viewing of fine structures such as dendrite spines even out to the

distal tips of the neuron;

[0046]   Fig. 18A is an image showing the pathological appearance of a

fluorescently labeled neuron exhibiting photo-toxicity from excessive

illumination;

[0047]   Fig. 18B is an image showing similar pathological appearance in another

neuron; this neuron, however, was able to tolerate the same general level of

illumination for a four-fold greater duration than that for the neuron shown in Fig.

18A; this increased tolerance was achieved through partial masking of the bright

cell body using an embodiment of the present invention; and

[0048]   Fig. 18C illustrates the pattern of illumination with decreased excitation to

the bright cell body used in experiment illustrated in Fig. 18B.

DETAILED DESCRIPTION OF EMBODIMENTS

[0049]   Enabling examples of the present invention provided herein discuss an

epi-illumination fluorescent microscopy apparatus. However, the ordinarily

skilled artisan readily comprehends that the invention may be deployed in any

type of illumination system utilizing fluorescent microscopy, and/or in non-

microscopy applications involving fluorescent emissions. Those of skill in the art

readily comprehend that the instant invention may be deployed with any type of

stimulating light illumination system used for stimulating a sample to produce excited fluorescence and/or for confocal masking.

[0050]   Achieving optimal image quality using a CCD detector requires that the sample being viewed possess an emitted light signal intensity that is within the linear range of the CCD. The present invention achieves this goal by adaptively modulating excitation illumination intensity on a pixel-by-pixel basis using a high resolution spatial light modulator, such as an LCD (liquid crystal display) or a DMD (digital micro-mirror device). The degree of modulation may be saved and later retrieved, thereby allowing reconstruction of a quality image with a high degree of accuracy and with highly favorable signal-to-noise characteristics. The dynamic range of the signal to be recorded may first be compressed to remain within the distortion free range of the recording device. If desired, the dynamic range of the recorded signal can then be un-compressed for quantitatively accurate and distortion free playback.

[0051]   The phrase "pixel-by-pixel," as used herein, describes the ability of the instant invention to modulate excitation illumination on a point-by-point and/or an area-by-area basis, and may therefore include any range of a number of pixels, from the lowest degree of resolution to the highest. Further, the present invention does not require that a certain number of pixels at an illumination device correspond directly to the same number (or any certain number) of pixels at an imaging device.

[0052]   Fig. 3 illustrates an embodiment of the present invention as implemented

in a fluorescent imaging system. Similar features previously discussed in relation

to Fig. 1 are not re-discussed so as to avoid redundancy.

[0053]   As shown in Fig. 3, an imaging device 10 is used to observe a biological

sample 22 through the dichroic mirror 30. The imaging device 10 may be a

charged coupled device (CCD), an intensified camera, a complementary metal

oxide semiconductor (CMOS) camera, a video camera, a photodiode detector

array, or another imaging device. A light source 40 produces a light that is

transmitted to a spatial light modulator 60. Spatial light modulator 60 receives

feedback from the CCD 10 via feedback loop 62. The feedback provided by

feedback loop 62 allows for modulation of the excitation light at the modulator 60.

Modulation of the excitation light is achieved using computer 150, and includes

modulating in at least a grayscale manner and based on information provided

from at least the CCD 10.

[0054]   The phrase "grayscale manner" is used to describe the ability of the instant

invention to control the precise excitation illumination intensity of each individual

pixel in the modulator, thereby providing gradients of light intensity at each

individual pixel ranging from zero intensity to the highest intensity that the

modulator 60 is capable of. An embodiment of the present invention modulates at

least one of the spatial location of the illuminated pixels and the gray levels of the

modulator's (element 60) pixels to discriminately excite the sample being viewed

(such as sample 22 shown in Fig. 3). In an additional embodiment, both spatial

and grayscale modulation may be used. In yet a further embodiment, an all-or-none (binary) modulation may be used.

[0055]   While the high resolution spatial light modulator 60 may comprise an LCD, a digital micro-mirror device, or another light modulating device, the embodiment shown in Fig. 3 uses an LCD. In this embodiment, an input polarizer positioned on the same side of the LCD panel as the light source provides the LCD panel with a uniform, linearly polarized cone of light. Each pixel in the LCD panel can thus independently rotate the input polarized light to an angle dictated by a voltage addressed to that pixel. The exit polarizer positioned towards the specimen then absorbs the rotated light to an extent dictated by the degree of rotation to create an image of differing intensities. The input polarizer, the LCD of the transmissive type, and the exit polarizer are illustrated as a single device in this application.

[0056]   Spatial light modulator 60 can thereby transmit a modulated excitation light 70 which is of the wavelength reflected by the dichroic mirror 30 onto the sample 22, where a fluorescent marker in the sample 22 is stimulated. The sample 22, thus excited, emits a fluorescent emission which is of a wavelength permitted to pass through the dichroic mirror 30, and which is recorded by the imaging device 10.

[0057]   In contrast to the device of Fig. 1, the sample 22 of Fig. 3 may be adaptively and discriminately illuminated with varying amounts of modulated excitation light 70 on either of, or any combination of, a spatial, a binary, and

grayscale basis, thereby allowing a viewer to alter the ultimate amount of
emission light produced in various portions of the sample. Modulation is based
on feedback information provided by the feedback loop 62 and is controlled by
computer 150. Alternatively, or in conjunction with the feedback, monitor 151
and graphical user interface 152 provide users with the ability to manipulate the
computer 150 to thereby control the modulation of modulator 60 through the LCD
controller 140.

[0058]   The modulator 60 may be controlled by the computer 150 through the
LCD controller 140 in a completely automated fashion wherein information
received from the CCD 10 automatically drives the type of modulation instruction
provided to modulator 60. For instance, if the sample 22 emits fluorescence near
the upper limit of the CCD's effective range of detection, the computer 150 is
capable of modulating the modulator 60 in a grayscale manner to lessen the
intensity of the stimulating light reaching the portions of the sample 22 which are
emitting near the upper limit of the CCD's effective range of detection, thereby
lessening the intensity of the fluorescence emitted by the sample 22.

[0059]   Further, the computer 150 provides the function of modulating the
modulator 60 in a semi-automatic fashion. For instance, fluorescent emissions
detected at the CCD 10 may be displayed on the display 151, along with potential
patterns of suggested modulation based upon prior viewing of particular types of
samples or based upon patterns of detected fluorescence as processed by an
algorithm within computer 150. Additionally, the computer 150 provides the

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function of modulating the modulator 60 in a fixed pattern, regardless of the nature of the sample being viewed. For instance, the modulator 60 may be instructed to emit a rectangular field iris or a flexible field iris of any shape. Users are provided the option of manipulating any of the above examples in regards to space, shape, and intensity of the illuminating light through use of the computer 150 and the graphical user interface 152. The present invention's features of automated and semi-automated control of the excitation light modulator 60 are further discussed below in relation to Figs. 11A – 11C.

[0060]   The degree of modulation implemented at each pixel of the spatial light modulator 60 is capable of being stored in a memory which can subsequently be retrieved to reconstruct a quantitatively accurate and distortion free image of the specimen. Reconstruction can be carried out by the computer through multiplication of the intensity recorded by the detector 10 with the feedback attenuation sent to the corresponding pixel on the spatial light modulator 60. Additionally and/or alternatively, reconstruction can be carried out by the computer through multiplication of the intensity recorded by the detector 10 with the feedback attenuation sent to the corresponding pixel, in conjunction with an optical transfer function (optical transfer functions are discussed herein in relation to Figs. 11A – C) of the spatial light modulator and the illumination optical system. Further, reconstruction can also be carried out through use of a user input in conjunction with the previous.

[0061]   Figures 4A – 4F illustrate a comparison of the emission images resulting

from prior art excitation illumination systems (*e.g.*, the prior art illumination

system of Figure 1) in comparison with an embodiment of the present invention

(*e.g.*, the embodiment of the invention depicted in Figure 3). Figures 4A – 4F

each include images captured from the same two spheres, 80 and 90 (sphere 80

fails to be depicted in Fig. 4A for reasons discussed below).

[0062]   Figure 4D is a graph corresponding to the emission brightness produced

by the two spheres 80 and 90 as shown by the image detected by a CCD in Fig.

4A. Conventional wide-field microscopy methods utilizing a scientific grade 12-

bit CCD camera (Photometrics SenSys 1401™) can barely detect the faint sphere

80 at the same time that the bright sphere 90 has already saturated the pixels of

the imaging device (as shown in Fig. 4A). This conventional method of

producing an image relates to the prior art system depicted in Figure 1, for

instance. As shown in Fig. 4A, the sphere 80 is not yet properly excited so as to

be properly imaged, while the sphere 90 has reached sufficient exposure of

excitation light so as to reach saturation. These results are depicted in the graph

of Fig. 4D, where sphere 80 possesses a negligible excited fluorescence emission

in comparison to the excited fluorescence emission of the sphere 90.

[0063]   Figure 4E is a graph corresponding to the excitation illumination

produced by the two spheres 80 and 90 as shown by the image detected by a CCD

in Fig. 4B. This conventional method of producing an image relates to the prior

art system depicted in Figure 1, for instance. When the excitation exposure is

increased to image the faint sphere 80, the image of the bright sphere 90 becomes grossly distorted (as shown in Fig. 4B). Notice that the bright sphere 90 appears falsely larger than its actual size. Sphere 80 and 90 are actually the same size, but exhibit differing brightness. Because the pixels surrounding the sphere 90 have become saturated, electron spillover has spread to neighboring pixels and post-acquisition image processing cannot be used to determine the true dimensions.

[0064]   Figure 4F is a graph corresponding to the emission illumination produced by the two spheres 80 and 90 as shown by the image detected by a CCD in Fig. 4C. Both the graph of Fig. 4F and the image of Fig. 4C have been created from an image and data resulting from the use of an embodiment of the present invention. In this embodiment, a high resolution spatial light modulator such as element 60 in Fig. 3 is used to provide adaptive and discriminate feedback-controlled excitation illumination.

[0065]   For example and referring to Figure 3 in addition to Figures 4C and 4F, an imaging device 10 takes an initial image of a sample 20 excited by modulated excitation light 70 from the high resolution spatial light modulator 60. If the resulting image is undesirable (e.g., if the image is similar to that shown in Figs. 4A, 4B, 4D, and/or 4E), the excitation light may be modulated to produce a more qualitatively pleasing image. For example, in a non-limiting, enabling embodiment of present invention, the sample 20 is stimulated with excitation light 70, wherein the excitation light 70 is modulated to be an inverted illumination of the sample's previously detected fluorescent emissions.

[0066]   "Inverted," as used herein, describes the application of a greater intensity

of excitation light to those points in a sample that exhibit fluorescent emittance

properties of lesser luminance and a lesser intensity of excitation light to those

points in a sample that exhibit fluorescent emittance properties of greater

luminance. Stated differently, bright pixels (as detected at CCD 10) are converted

to dark pixels for purposes of modulating the excitation light 70. As used herein,

"excitation" is generally used to describe the light deriving from the light source

40, and "emitted fluorescence" or "emission(s)" is generally used to describe the

light fluorescing from the sample once excited. Other terms could be employed,

however, to possibly include the use of "stimulated" instead of "excitation" and/or

"excited" or "excited emission" instead of "emission" or "emitted fluorescence."

[0067]   Using the initially detected fluorescent emission as feedback for an inverse

level of excitation light provides the qualitatively pleasing image shown in Figs.

4C and 4F, for example. The ordinarily skilled artisan readily comprehends that

the excitation light does not need to be a linearly inverse of the detected

fluorescent emission to derive the benefit of the invention, however. Indeed, the

excitation light may be modulated to any lesser or greater degree to derive a

qualitatively pleasing image, depending on the precise requirements of the sample

being viewed through use of the invention's ability to cast light from the

modulator 60 in a grayscale manner.

[0068]   Figs. 17A and 18A are images taken of fluorescently labeled neurons

using conventional excitation illumination methods. The image of Fig. 17A

illustrates a live neuron expressing green fluorescent protein (GFP) taken using a

conventional homogenous epi-illumination system. Note that the brightness of

the cell body has saturated the image as detected by the imaging device, creating

distortions to the size of the cell body that cannot be corrected, but that the distal

dendritic branches are barely detectable. Furthermore, the glow from the bright

cell body has created a false relative brightness of proximal dendrites compared to

distal dendrites.

[0069]   In comparison, the image of Fig. 17B was taken of the same neuron but

using the excitation illumination modulation of the instant invention. Note that

the cell body is not saturated and therefore does not appeal to be (falsely) larger

than it actually is. The fine structures of the dendrites such as the dendrite spines

can be fully appreciated out to the most distal tips. Furthermore, the false

brightness of the proximal dendritic processes shown in Fig. 17A has been

eliminated.

[0070]   Fig. 18A illustrates the typical pathological appearance of a fluorescently

labeled neuron associated with photo-toxicity due to excessive exposure to

excitation illumination using a convention illumination system. Note the

formation of bead-like dilatations along axonal processes and the formation of

blebs at dendritic branch points. Without masking of the bright cell body the

previously noted pathological changes appear quite rapidly.

[0071]   Contrastingly, Fig. 18B illustrates the appearance of another neuron

wherein the cell body has been partially masked using the excitation modulator of

the instant invention. This cell was able to tolerate a four-fold increase in exposure time at the same overall intensity to reach a comparable degree of injury.

[0072]   Fig. 18C illustrates the pattern of illumination with cell body masking that allowed the neuron to tolerate greater light exposure using an embodiment of the instant invention.

[0073]   The spatial light modulator 60 shown in Figure 3 may comprise a liquid crystal device (LCD), a digital nano- or micro-mirror device (DMD) and/or a nano- or micro-electro device. In an enabling embodiment and for transmissive displays, an LCD panel is placed between two polarizers. The incoming polarizer presents the LCD panel with a uniform, linearly polarized cone of light. Each pixel in the LCD panel can independently rotate the input polarized light to an angle dictated by the voltage addressed to that pixel. The outgoing polarizer then absorbs the rotated light to an extent dictated by the degree of rotation to create an image of differing intensities.

[0074]   The inventor has found that while either a wire grid or polymer polarizer may be employed, desired qualities in choice of polarizer include heat tolerance, high extinction for cross polarized light, and high transmission for parallel polarized light. A specific polarizer envisioned for the invention's application is a heat tolerant and high contrast polymer polarizer (for example, certain polarizers made by Polatechno, Inc., in Japan; another source of high-quality polarizers includes the company Moxtek, Inc.).

[0075]   It is known that LCD contrast can decrease with increasing temperature.

The "clearing temperature" (the temperature at which an LCD panel looses

contrast) for most LCDs is approximately 85 degrees C. While prototypes of the

instant invention did not encounter the specific problem of the LCD reaching a

temperature at or above 85 degrees C, the inventor remained ready to address this

potentiality with known cooling methods, for example, a cooling fan to provide a

remote fan-driven air stream.

[0076]   Additional steps used to optimize LCD contrast include minimizing

reflection off the exit polarizer. For instance, any wire grid polarizer which is

high reflective should only be used as the input polarizer and not as an exit

polarizer. An additional LCD compensation polarizer may be placed on the exit

side of the LCD panel to improve image contrast.

[0077]   Advantages of using an LCD in the present invention include the fact that

LCD panels are relatively thin, and the further fact that the common x-y physical

dimensions of LCD panels typically match the allowable dimensions of common

field diaphragms used in typical fluorescence microscopes. Consequently, the

LCD assembly of the instant invention can be positioned simply and directly

within the field diaphragm slot in the epi-illumination unit of the typical

fluorescence microscope, without needing to alter the optics of the typical

microscope. Furthermore, LCD panels are relatively inexpensive.

[0078]   A digital micro-mirror device (DMD) may also be used as the high

resolution spatial light modulator 60 as shown in Figure 16. DMDs are also

referred to as DLPs (digital light processors) by the main manufacturer of these devices (Texas Instruments). While the current trend is to label the noted digital micro-mirror devices as being "digital" and "micro," the ordinarily skilled artisan readily comprehends that such devices could also be analog, and/or smaller than micro (such as nano-sized) or even larger than micro-sized.

[0079]   Current DMD technology differs from LCD technology by producing an 'all-or-none' signal for each pixel. By way of explanation, DMD technology micro-mirrors are connected to pivots fabricated as a large, closely packed array. Each micro-mirror can be independently addressed and controlled so as to be oriented in one of two directions (if not more than two directions). In an "on" position, light is reflected into the microscope's imaging path, and in the 'off' position light is directed away from the microscope's imaging path.

[0080]   An exemplary embodiment of the present invention comprising the slider 120 containing the LCD panel and the LCD controller is shown in Fig. 5A. The LCD is connected to the slider by a ribbon cable 110. As shown in Fig. 5B, the LCD 60 (mounted in the slider 120) is capable of being snugly positioned in the field diaphragm slot 130 of a common microscope (e.g., the inventor utilized a Nikon upright microscope's epi-fluorescence unit). Other LCD panels could be used in similar fashion with other common microscopes, such as Olympus microscopes, Leica microscopes, or Zeiss microscopes. The spatial light modulator may be placed in the epi-illumination paths of either upright of inverted microscopes.

[0081]   Fig. 6 illustrates a representative LCD panel 66 that can be used as the

active element in a LCD based spatial light modulator 60. Towards the right side

of Fig. 6 is a representative DMD 67 that could be used for in a DMD based

spatial light modulator.

[0082]   Fig. 7 illustrates mechanical and electrical hardware for implementation of

an embodiment of the invention (for ease of understanding, like components are

labeled identically to prior figures). As shown in Fig. 7, a CCD 10 interfaces with

a computer 150 via a frame grabber card within the PC. Image processing

software within the computer 150, with or without user input, analyzes a CCD

image and computes a corrective modulation for the spatial light modulator 60 to

thereby discriminately excite the sample being viewed. The CCD 10 takes its

image from a sample being viewed by a microscope, for instance (the microscope

is not shown).

[0083]   The computer 150 may determine if the image taken is over or under

exposed. Alternatively, a user may enter values to drive the excitation light

modulator 60 to produce a discriminating excitation illumination light based on

knowledge of the sample currently under observation. A previously stored set of

values may also be retrieved and supplied as control signals to the modulator.

The camera 10, frame grabber and computer 150 may be off-the-shelf items. The

computer 150 includes a graphical user interface (GUI) and a plug-and-play

"driver" for connecting to the LCD controller 140. The LCD controller 140

drives the LCD panel serving as the spatial light modulator 60.

[0084]   Fig. 8 illustrates exemplary sub-components of the Liquid Crystal Device

controller 140.   The controller 140 may connect to the computer 150 via a

Universal Serial Bus (USB) interface. A microcontroller 170 provides the

controller's USB connection. The corrective image is received by the

microcontroller 170 from the computer 150.

[0085]   The computer receives the original image from the CCD 10 potentially

using any one of many commercially available image acquisition software and

computer interface. One example of commercial image acquisition software is

IPLab™ from Scanalytics. The computer may utilize additional software

(described below) to interface with the LCD controller. The computer, image

acquisition software, and/or user perform a qualitative and quantitative analysis of

the original image to determine the most-likely modulation scheme for driving the

spatial light modulator 60 to produce a qualitatively pleasing image (additionally

and/or alternatively, a user may enter modulation characteristics desired for a

particular sample). The modulation scheme produced by the computer 150 may

be stored in a frame buffer 180 of the controller/projector 140. The frame buffer

180 is coupled to a display controller 190. The display controller 190 generates

horizontal and vertical synchronization signals for the LCD panel 60 and

transmits the contents of the frame buffer 180 to the panel 60.

[0086]   Software useful for implementing the present invention includes a

graphical user interface (GUI) which provides manual functionality of alignment

and image processing.

[0087]   The alignment of the LCD image mask in the illumination path is critical

for the precise masking of structures in any microscope image plane. The goal is

to achieve a precise co-registration of LCD pixels with the corresponding pixels

of the electronic image sensor. At least three parameters require adjustments.

First, the location of the center of the spatial light modulator projected on the

target specimen must also correspond to the center location of the image detector.

Second, the size of the pattern projected onto the target specimen must correspond

to the size of the pattern detected by the image sensor. Thirdly, the orientation of

the projected pattern must correspond to the orientation of the pattern detected by

the image sensor. A fourth parameter, optical distortions, may require adjustment

if significant optical distortion exists between the image forming and specimen

illumination optical paths. Significant optical distortions are not usually observed

in the center of the field of view for high quality scientific microscopes.

[0088].   The alignment and co-registration of the image detector and spatial light

modulator pixels may utilize mechanical positioning of the spatial light modulator

and/or image detector. However, the preferred method for routine alignment and

co-registration is to electronically control the output of the spatial light modulator

either in a manual process or an automated algorithm. In the manual alignment

process a target pattern is sent to both the spatial light modulator and the image

acquisition software. The pattern is projected as an overlay over the image

acquired by the electronic image sensor. The pattern send to the spatial light

modulator is projected onto a smooth flat target. In this manner the image

acquisition software can display both the expected pattern and the pattern actually

projected onto the target. The user can then manually adjust the x,y position of the

pattern, the magnification, and rotational orientation of the spatial light modulator

via the LCD/DMD controller. Once adjusted the settings can be saved by the

software/computer for subsequent use until there is a change to the optics or the

slider position. An example of a target pattern may consist of a crosshair located

at the center and brackets located just within the four corners of the electronic

image sensor. If correction of optical distortion is required, the target pattern will

contain grid patterns that can best detect the optical distortion. A morphing

algorithm will be used to correct the optical distortion.

[0089]   Figs. 9A and 9B illustrate an example of an automated algorithm for

optimal alignment and co-registration of the LCD image to the CCD. Dots 210a

and 220a illustrate the two reference points needed to provide a two-dimensional

alignment. The two points 210a and 220a are projected by the LCD excitation

light modulator 60 and captured by the CCD 10, as shown in Fig. 9A. The

location of the two circles is predetermined on the CCD camera to provide an x-y

coordinate "target."

[0090]   Fig. 9B is an overlay of the LCD projected image captured by the CCD

and the expected target locations of the LCD projected image on the CCD,

showing how dots 210a and 220a do not quite line up with dots 210b and 220b

(presumably this is because an objective has been changed, or because the slide

sample was adjusted or for any reason resulting in the image not being viewed in

an original state). An automated algorithm is capable of calculating the

necessary translation, rotation, and magnification of the calibration points to be

used to generate LCD masks with a co-registration of the LCD mask and the CCD

such that dots 210a and 220a align with dots 210b and 220b, respectively.

[0091]   A task often utilized in fluorescence microscopy is the process of masking

the illumination/excitation area in a field of view. This functionality is of interest

for several reasons. First, when a very bright structure in an image field

dominates the dynamic range of the CCD camera, darker and smaller structures of

interest become barely visible and thus difficult or impossible to analyze. In this

situation, an investigator often limits the area of excitation in the field of view to

exclude the bright structure.

[0092]   Second, within a field of view it may be of interest to continuously image

a structure while minimizing excitation of nearby structures. This situation often

arises when an investigator is performing live cell imaging on one cell or part of

one cell in a field of other live calls and wants to minimize the exposure of the

other cells and parts of the same cell. Figs.18A - C illustrate the effects of

phototoxicity during live cell fluorescence imaging.

[0093]   Both problems are often addressed with commercially available field iris

insets, which limit the excitation field to a defined field of view. These

mechanical barriers are of a single shape (usually a circle or a square) and size,

and are in a fixed position in the excitation path, making their use limited and

inflexible. Furthermore, it is often desirable not to block illumination completely

to the neighboring structures. Even existing electronically addressable field stops

can not provide precise graded illumination.

[0094]   Figs. 10A-D illustrate an embodiment of the present invention including

an interface that allows a user to custom-design a Field Iris Mask (FIM). Figures

10A-D are theoretical set of masks created with either a transparent (shown by

Figs. 10 B and 10D as representing no density) or blocking (100% density, as

shown by Figs. 10A and 10C) background pallet, in which a rectangle, or a circle

or free-form shape is defined. Areas of interest (AOI) are user-defined through a

portion of a GUI software implemented in a computer 150 (such as the computer

150 shown in Fig. 7). A user chooses from AOI tools, which contain pre-set

shapes (e.g., a circle, a square, a rectangle and/or an ellipse) or a freeform tool

which is interactively created via mouse interaction.

[0095]   Because of differences in samples and in the desired structures that the

user may wish to bring out, the instant invention provides CCD/LCD contrast

transfer functions or density transfer functions that can be used to provide graded

level of feedback illumination. These functions may include linear, logarithmic,

threshold, or user-defined density transfer functions.

[0096]   Examples of logarithmic functions are illustrated in Figs. 11A-C. In these

examples, an image was collected on a 12 bit CCD camera (4096 level resolution)

with a maximum image intensity of 20,000 units. Figs. 11A and 11B illustrate the

detected intensity of the CCD camera (y-axis) versus the optical intensity of the

image(x-axis). The detected intensities are shown as solid lines. The unmasked

theoretical object intensity is shown as a dotted line in Fig. 11A. Fig. 11B shows

a logarithmic density transfer function which provides either of an automated or

semi-automated control of the excitation light modulation. Most cellular

structures saturated at a value of 4096 as shown by the horizontal solid line in the

top panel of Fig. 11A and as white on the gradient scale in the center panel of

Fig.11A.

[0097]   A logarithmic density transfer function is shown in the column of Fig. 11B.

The top panel of Fig. 11B shows the final correlation between the un-masked

object intensity and the masked intensity that falls within the dynamic range of

the CCD camera. The gray scale is shown in the center panel. The transmittance

of the mask for this object using the logarithmic density transfer function is

shown in the bottom panel of Fig. 11B. As stated previously, in various

embodiments of the instant invention, the computer 150 and the controller 140

(such as those shown in Fig. 3) are capable of implementing the logarithmic

functions shown in Figs. 11 A – C in either of an automatic or semi-automatic

fashion based on the fluorescent emissions detected by the imaging device 10.

[0098]   Figs. 17 B illustrate the benefits of the current invention on image quality

as compared to the conventional system depicted by Fig. 17A. Specifically, the

distortion in size created by pixel saturation at the bright cell body is eliminated in

Fig. 17B as compared to Fig. 17A.   Further, the distortion in the relative

brightness of structures of near by to distant structures is eliminated in Fig. 17B as

compared to Fig. 17A. Additionally, the signal-to-noise ratio of dim structures is also improved in Fig. 17B as compared to Fig. 17A.

[0099]   Figs. 18A - C illustrate the ability of the present invention to decrease the rate of photo-toxicity in live cell imaging. Fig. 18A illustrates an unmasked live cell with associated photo-toxicity due to a bright cell body. Figs. 18B and 18C illustrates a partial masking of the bright cell body containing large amount of fluorescent dyes, thereby increasing the allowable illumination time by greater than four-fold before appearance of pathological changes on axonal and dendritic processes.

[00100]   Another common problem in fluorescent microscopy is an out-of-focus blur causing poor image quality. Confocal microscopy heretofore has not been easily implemented. There may be situations where an image could be greatly improved if only bright out-of-focus blur could be eliminated from a specific plane. Because of the confocal nature of the instant invention's spatial light modulator controlled excitation illumination system, it is possible to attenuate out-of-focus blur that occurs in a conventional wide-field microscope as described below.

[00101]   Figs. 12A and 12B illustrate the invention's technique of masking an out-of-focus signal. Fig. 12A illustrates a field diaphragm plane that is conjugate to the image plane. Patterns projected from the spatial excitation light modulator 60 positioned at the field diaphragm plane are confocally projected (masked) onto the sample being observed. If the axial position of the spatial excitation light

modulator 60 is shifted, the masking action also shifts by a distance proportional

to the ratio of the focal length of the objective and tube lens. This principle is

shown by Figs. 12A and 12B, wherein a pair of small fluorescent spheres was

placed in one plane and a pair of larger spheres was placed in another plane.

[00102]   Fig. 12A illustrates the expected image when the focus is on the small

spheres. Note that the out-of-focus spheres make the image in Fig. 12A

practically impossible to read. In contrast, the instant invention, by first taking an

in-focus image of the fluorescent emittance of the two larger spheres, inverting

the excitation illumination at the modulator 60 as compared to the detected

fluorescent emittance and shifting the modulator 60 by an appropriate distance,

provides the advantageous result of selective masking the two out-of-focus

spheres without changing the image of the in-focus spheres (as shown in Fig.

12B). The result is a qualitatively and quantitatively pleasing image.

[00103]   The concepts related in Figs. 12A and 12B are also shown in Fig. 15.

Similar features previously discussed in relation to Figs. 3 and 12A and 12B are

not re-discussed so as to avoid redundancy.

[00104]   In Fig. 15, the spatial light modulator 60 is capable of movement along the

illumination axis. The modulator 60 may be moved by any of sliding wedges,

piezo electric actuators, a stepper motor actuator, and/or other

mechanical/electrical/pneumatic systems. The preferred method is through the

coupled rotation of multiple threaded posts on which the spatial light modulator is

mounted. The dotted line shown by numeral 42 represents the previous field

diaphragm plane prior to movement of the modulator 60 to a different plane shown by the dotted line at numeral 43. Movement of the modulator 60 allows for confocal image masking such as that described in relation to Figs. 12A and 12B. Furthermore, in very thin specimens the thin dark grid pattern inherent to LCDs will appear in the acquired image. Therefore, it may be advantageous to move the position of the spatial light modulator slightly away from the conjugate image plane in order to defocus the fine grid patterns of the LCD.

[00105]   In most modern fluorescent microscopes the lens for image formation 26 is designed to be different from the lens 28 for specimen illumination. The previous can result in markedly different magnification of patterns located at image plane 42 and the conjugate image plane at the detector 10. Therefore, a single LCD pixel typically corresponds to 16 or 25 CCD pixels, which is not an optimal utilization of LCD resolution. The solution is to increase the focal length of the epi-illumination tube lens 28. The change in focal length of the epi-illumination lens is represented by the dotted circle enclosing lens 28 and 29. The increase in the focal length of this tube lens is particularly important for large size spatial light modulators. With any significant change in the tube lens the spatial light modulator will also need to be repositioned to a plane close to its new conjugate image plane 43.

[00106]   Fig. 15 also shows an alternative arrangement of the light source 40 being reflected by mirror 44 to the light modulator (an LCD in Fig. 15) through condenser 32.

[00107]   Fig. 16 illustrates a microscope system similar in nature to that shown in Fig. 15, but includes a DMD instead of an LCD for the modulator 60. Similar features previously discussed in relation to Figs. 3 and 15 are not re-discussed so as to avoid redundancy. In contrast to LCD of the transmissive type, a DMD spatial light modulator 60 creates image patterns by deflecting light at each pixel. In response to an ON input voltage, the mirror deflects the incident light into the clear aperture of the illumination tube lens 28. The path of the light deflected in the ON state is represented by the dotted line in Fig. 16.In response to an OFF input voltage, the mirror deflects the incident light outside of the clear aperture of the lens 28 (outside of cone 70)..

[00108]   The ability to modulate the DMD 60 to achieve a grayscale pattern is different than that of an LCD. Whereas the LCD achieve gray levels by graded rotation of the incident polarized light, a DMD achieves gray levels by "flickering" between the ON and OFF modes of the individual micro-mirrors. An example (incorporated herein by reference) of grayscale levels using DMD technology is described by Larry J. Hornbeck in *Digital Light Processing for High-Brightness, High-Resolution Applications* (available at www.vxm.com/TIDLP.html). In this white paper, Hornbeck describes achieving grayscale brightness levels by modulating the binary pulse width of the incident light. By rapidly moving the micro mirrors from an "on" position to an "off" position multiple times in an integration period of the electronic detector 10, a certain level of grayscale fluorescence can be realized.

[00109]   For example, using a Texas Instruments micro mirror device, a user can

expect a switching time from "on" to "off" of about 15 microseconds. The typical

integration time for a CCD to capture a fluorescent image is approximately

between 1 ms to 1000 ms. A grayscale is determined by the percentage of time

that the micro mirror is in the "on" state versus the "off" state during the

integration period.

[00110]   A typical DMD accepts electrical words which are used to represent

grayscales. These words are encoded in binary form. For example, a 10-bit word

may define 1024 gray levels. A 4-bit word will have a time duration with relative

values of 1, 2, 4 and 8 within the integration time window.

[00111]   A further embodiment of the present invention provides the ability to

calibrate for contrast, resolution and aberration, as shown in Figs. 13A and 13B.

In Fig. 13A, a command image is taken of a set of white bars of differing

brightness as against a black background. These bars are then projected onto a

flat surface, such as a front surface mirror. The grid pattern on the white bars

represents the fine grid that separates individual LCD pixels. The ability to detect

these grids provides a measure of spatial resolution. The straightness of the grids

provides a measure of potential aberration. Further, as shown in Fig. 13B, a

vertical line scan across just one strip is used to measure the absolute intensity

detected by each CCD pixel, including resolution and contrast.

[00112]   Figs. 14A and B are schematic representations of exemplary, non-limiting

embodiments of how a modulator may be moved to change the magnification of

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the modulated excitation light. Skilled artisans readily comprehend that a

modulator may be re-positioned using other mechanical, electrical, and/or

additional repositioning means.

[00113]   Fig. 14A is a top-view and Fig. 14B is a side view of a Liquid Crystal

Device 60 attached to a sliding fork 230. By adjusting the sliding fork 230 in the

directions of arrows 240, the LCD moves to adjust to differing physical

dimensional placement, thereby adjusting the focus of any projected image.

[00114]   The above-identified invention may be embodied in a computer program

product, as will now be explained.

[00115]   On a practical level, the software that enables a computer system to

perform the operations described above may be supplied on any one of a variety

of media. Furthermore, the actual implementation of the approach and operations

of the invention are actually statements written in a programming language. Such

programming language statements, when executed by a computer, cause a

computer to act in accordance with the particular content of the statements.

Furthermore, software that enables a computer system to act in accordance with

the invention may be provided in any number of forms including, but not limited

to, original source code, assembly code, object code, machine language,

compressed or encrypted versions of the foregoing, and any and all equivalents.

[00116]   One of skill in the art will appreciate that "media", or "computer-readable

media", as used here, may include a diskette, a tape, a compact disc, an integrated

circuit, a ROM, a CD, a cartridge, a memory stick, a card, a remote transmission

via a communications circuit, or any other similar medium useable by computers known now or developed hereafter. For example, to supply software for enabling a computer system to operate in accordance with the invention, the supplier might provide a diskette or might transmit the software in some form via satellite transmission, via a direct telephone link, or via the Internet. Thus, the term, "computer readable medium" is intended to include the entire foregoing and any other medium by which software may be provided to a computer.

[00117]   Although the enabling software might be "written on" a diskette, "stored in" an integrated circuit, or "carried over" a communications circuit, it will be appreciated that, for the purposes of this application, the software will be referred to as being "on" the computer readable medium. Thus, the term "on" is intended to encompass the above and all equivalent ways in which software is or can be associated with a computer readable medium.

[00118]   For the sake of simplicity, therefore, the term "program product" is thus used to refer to a computer readable medium, as defined above, which bears, in any form, software to enable a computer system to operate according to the above-identified invention. Thus, the invention is also embodied in a program product bearing software which enables a computer to perform according to the invention.

[00119]   The invention is also embodied in a user interface invocable by an application program. A user interface may be understood to mean any hardware, software, or combination of hardware and software that allows a user to interact

with a computer system. For the purposes of this discussion, a user interface will

be understood to include one or more user interface objects. User interface

objects may include display regions, user activatable regions, and the like.

[00120]   As is well understood, a display region is a region of a user interface

which displays information to the user. A user activatable region is a region of a

user interface, such as a button or a menu, which allows the user to take some

action with respect to the user interface.

[00121]   A user interface may be invoked by an application program. When an

application program invokes a user interface, it is typically for the purpose of

interacting with a user. It is not necessary, however, for the purposes of this

invention that an actual user ever interact with the user interface. It is also not

necessary, for the purposes of this invention, that the interaction with the user

interface be performed by an actual user. That is to say, it is foreseen that the user

interface may have interaction with another program, such as a program created

using macro programming language statements that simulate the actions of a user

with respect to the user interface.

[00122]   The previous description of embodiments is provided to enable a person

skilled in the art to make and use the present invention. Various modifications to

these embodiments will be readily apparent to those skilled in the art, and the

generic principles and specific examples defined herein may be applied to other

embodiments without the use of inventive faculty. For example, some or all of

the features of the different embodiments discussed above may be deleted from

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the embodiment. Therefore, the present invention is not intended to be limited to

the embodiments described herein but is to be accorded the widest scope defined

only by the claims below and equivalents thereof.